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Contents

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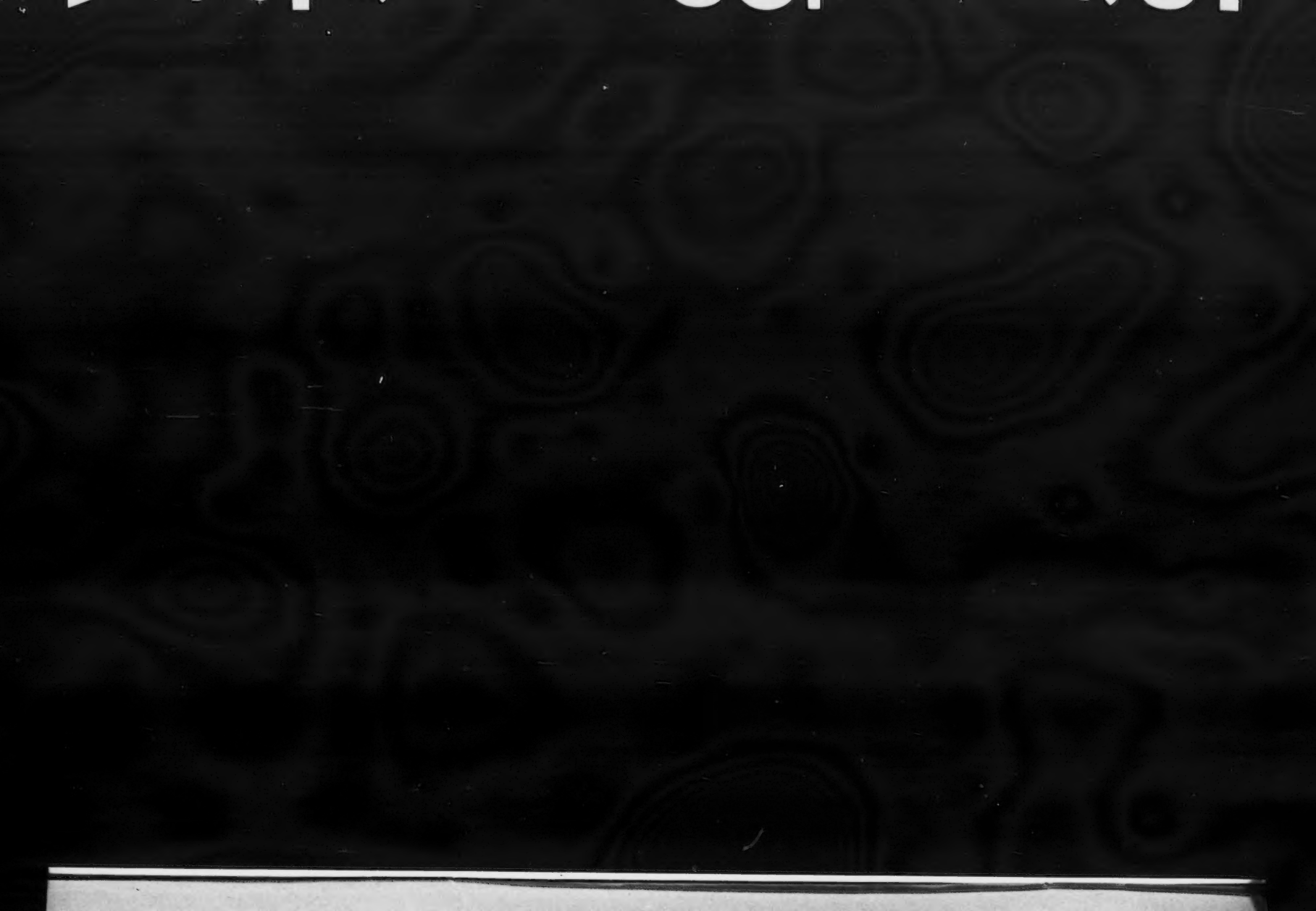

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The Canadian Journal of Research is at present published in six sections, A to F. Starting with January 1, 1951, these sections will be published as separate journals under distinctive names and the designation Canadian Journal of Research will no longer be used. The present names and the corresponding new names are as follows:

Present Name	New Name
Canadian Journal of Research, Section A (Physical Sciences)	Canadian Journal of Physics
Canadian Journal of Research, Section B (Chemical Sciences)	Canadian Journal of Chemistry
Canadian Journal of Research, Section C (Botanical Sciences)	Canadian Journal of Botany
Canadian Journal of Research, Section D (Zoological Sciences)	Canadian Journal of Zoology
Canadian Journal of Research, Section E (Medical Sciences)	Canadian Journal of Medical Sciences
Canadian Journal of Research, Section F (Technological Sciences)	Canadian Journal of Technology

In order to preserve continuity the present sequence of volume numbers will be retained, and in each case the volume for 1951 will be Volume 29.

The subscription rates for the Journals will remain as at present.

Canadian Journal of Research

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VOL. 28, SEC. D. OCTOBER, 1950 NUMBER 5

THE EFFECT OF FOREST SUCCESSION UPON THE QUANTITY AND UPON THE NUTRITIVE VALUES OF WOODY PLANTS USED AS FOOD BY MOOSE[1]

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Abstract

The moose, *Alces americana*, in British Columbia reaches its greatest populations in the young second growth forest areas. Numbers decrease as the forest approaches its climax stage. The decline in population is known to be mainly the result of malnutrition.

The present study of three stages in forest succession growing under virtually identical conditions of soil and climate has concerned itself with quantity of available palatable browse; carotene and ascorbic acid content of available palatable and unpalatable trees and shrubs; and with determination of values for moisture, protein, carbohydrate, ether extractives, and total mineral content. Most of the analyses are confined to the winter dormant period.

It is determined that the forest changes studied involve a reduction in quantity of palatable browse to about one-third; that there is an increase of carotene values and possibly of total mineral content in the vegetation on more advanced forest areas, but that in ascorbic acid content, ether extractives, total carbohydrates, and proteins the vegetation upon the younger forest areas is superior to that on the older areas.

It is concluded, therefore, that the declining carrying capacity noted in a forest approaching its climax stage results from decreases in both the quantity and quality of food produced.

It is further concluded that the most desirable winter range for moose is one upon which there is a variety of palatable species, predominantly in an early stage of growth, but with an intermixture of older forest stands bearing palatable coniferous trees.

Introduction

The moose, *Alces americana*, in British Columbia reaches its greatest populations in the young second growth forest areas. In the central part of the Province great forest fires accompanied early settlement, fires that removed the climax forest of Engelmann spruce (*Picea Engelmanni*) and left vast tracts of land clothed in one or another of the early successional stages of forest regeneration. Of paramount importance to the moose population is the plant association dominated by aspen (*Populus tremuloides*) and willow (*Salix* sp.).

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As this pioneer association matures it is gradually replaced by the climax forest of the region with the entire cycle occupying about a century. From the standpoint of the capacity of the land to support ungulate game mammals the cycle from denudation to climax is equally spectacular.

It is quite obvious that the pioneer plant community five or six years after its establishment offers an abundance of deciduous browse. Later, as the aspens and many of the willows become taller and achieve height beyond the reach of moose the crown shading reduces the amount of forage available to the browsing ungulates. At the same time there is a steady encroachment of young trees of spruce and balsam (*Abies lasiocarpa*) that in turn overshadow even the aspens to the end that the forest canopy is eventually closed, with so little light penetrating to the forest floor that deciduous vegetation becomes almost eliminated and with it goes the capacity of the forest to support ungulate mammals. Accompanying this reduction in density of the deciduous shrub understory goes a change in the species composing it. There is a gradual reduction in the palatable species and increase in the unpalatable species of shrubs (Tables I and II, and Fig. 1).

A climax Engelmann spruce forest supports moose and similar big game mammals only to the extent that lake shores, stream margins, or other clearings provide the necessary amounts of deciduous vegetation. Spruce, the dominant climax species, is unpalatable to browsing mammals, and alpine fir, though palatable and reasonably nutritious, is seldom present or available in appreciable quantities.

The contribution of the density of palatable vegetation to the carrying capacity of such a forest is then fairly obvious. However, carrying capacity is governed not alone by bulk of vegetation available, but is rather the product of bulk and quality. In other words, the important factor is total nutrients.

Our observations of moose on the different forest types led us to suspect that while mere shortage of sufficient *quantity* of suitable vegetation was probably the most important factor contributing to the starvation losses that we observed upon maturing forest areas, a deterioration in quality might also exist. It was accordingly decided to investigate the nutritive quality of the vegetation at different stages in forest succession. Earlier work, especially that by Einarsen (6), revealed the part played by reduced light in bringing about a lowered protein content of certain shrub and tree species used as food by deer and documented the consequences of this reduced protein content upon the deer population. It occurred to us that similar mechanisms might be operating to reduce the nutritive value of the woody plants used by moose at the same time that the normal processes of forest succession were reducing the total quantity of available browse.

In mountainous terrain in northern Canada, winter range and the condition of the food plants in winter are the ultimate factors limiting the carrying capacity of any region. Field observations upon moose have made it clear

that during the summer months the animals were in excellent nutritive condition. The winter months, however, often saw a gradual or rapid decline in physical condition of the moose apparently traceable to dietary deficiencies.

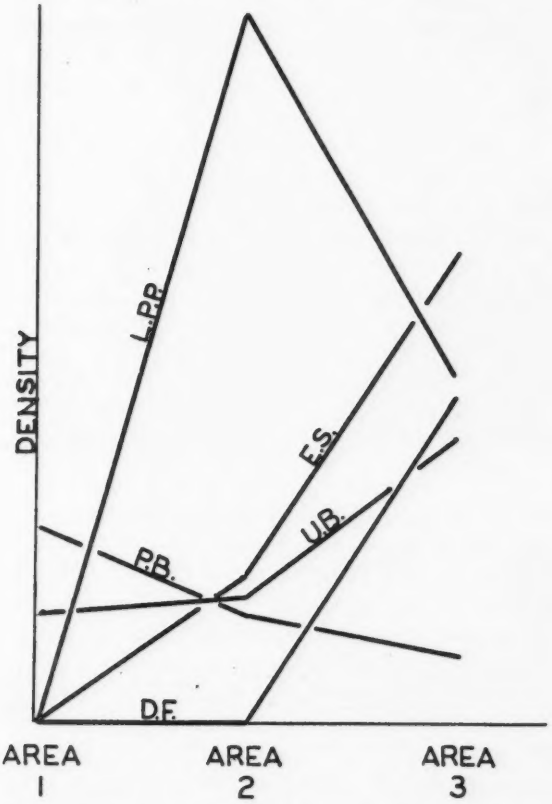

FIG. 1. *Changes in density of coniferous, palatable, and unpalatable deciduous browse accompanying forest ageing. All trees included 1 in. or more D.B.H.*

D.F.—Douglas fir; E.S.—Engelmann spruce; L.P.P.—lodgepole pine; P.B.—palatable browse; U.P.—unpalatable browse.

For this reason it was decided to concentrate our investigation of the chemical composition of the browse plants upon this critical period. In order to obtain an understanding of the nutritive quality of moose foods under different circumstances, three types of information were sought.

1. The relative values of the different species of shrubs and trees available.
2. The fluctuations or trends in quality through the winter months.
3. Changes in quality that accompany forest succession toward the climax.

Methods

Areas Selected for Study

Many studies of animal nutrition have shown that plants are quick to reflect local changes in soluble soil constituents and that the nutritive qualities of the same species may vary greatly from one site to another. In order to eliminate this possibility of error in our results we sought and found a region where different stages in forest succession occurred close together under virtually identical soil conditions.

The region chosen lies adjacent to the airport at Quesnel, B.C. Here, on a level glacial-outwash bench of apparently homogeneous structure we found three stages in the succession in close proximity.

The heavy conifer forest was cleared in 1942 for the purpose of setting out the air field. The trees were felled and the brush cut down and burned. The result has been to leave a sharp boundary between the remaining section of heavy forest and the adjacent cleared area. Only a small part of the clearing was paved for the actual landing strip. Between this and the forest is a strip 200 yd. wide and almost two miles long where there is now a healthy stand of deciduous seral trees and shrubs in marked contrast to conditions obtaining in the adjacent forest. South of the Barkerville road and immediately opposite the old forest a third, or middle, stage in succession was found intermediate between the other two. Its close proximity and apparently identical soil type rendered it suitable for comparative study. The new clearing, six years old when studied, was designated Area 1 (Fig. 3); the intermediate stage, Area 2 (Fig. 4), and the old forest, Area 3 (Fig. 5).

Ring counts on the oldest pines in Area 2 revealed that the area had last been cleared by fire between 20 and 30 years previous. Increment borings on several of the largest pines (*Pinus contorta*, a pioneer species) occupying Area 3 revealed the forest to be 70 years old or more. One very large tree contained 94 annual rings. All three areas were lightly used by moose at the time the study was made.

Floral Composition

As an essential preliminary to the studies of nutritive quality of the browse a careful appraisal of plant cover on the three areas was made.

Methods used were as follows:

Conifer density.—The technique consisted of establishing rectangular plots 100 yd. long and 2 yd wide. By means of a compass a 100 yd. chalk line was laid out and all conifer trunks within a yard on either side were recorded and the trees classified as to height and to D.B.H. Density is expressed in terms of numbers of trees in each height class up to 54 in. and beyond that in terms of numbers of trees in each of several classes as to diameter at breast height (54 in.).

Shrubs and deciduous trees.—The density of this group of plants was determined by the line interception coverage method. Ground coverage and height

FIG. 2. *Areas 1, 2, and 3. Quesnel, July 1948.*
FIG. 3. *Area 1. Note density of aspen due to vegetative reproduction.*

FIG. 4. *Area 2. Lodgepole pine and aspen predominant.*
FIG. 5. *Area 3. Lodgepole pine, Douglas fir, and spruce. Note the absence of tall shrubby union.*

classes of each species were recorded at interception with 100 yd. of chalk line placed by means of a compass in a predetermined direction.

Herbs.—The frequency of occurrence of herbaceous plants was obtained along the line laid out for study of shrub cover. A frame 1 ft. square was placed at intervals of three feet along the chalk line and the presence of the various species noted. In all cases samples were added until additional ones had no material effect upon the result.

Sampling for nutritive studies.—In taking the sample the operator walked across the area removing not more than one small branch from each tree or shrub encountered. When an adequate sample had been accumulated it was bundled, quick frozen, and shipped by air to the laboratory where it was either processed immediately or again frozen and kept frozen until needed. Collections were made only on days preceded by a 36 hr. period without precipitation.

Chemical Procedures

In the laboratory the current year's growth was trimmed from the twigs, chopped, dried to constant weight at 80°–90° C., run through a Wiley Mill with a 20 mesh screen, and then stored in sealed jars, along with a dehydrating agent.

For each sample the following determinations were made:

1. Total minerals: Expressed in terms of the residue from burning at 650° C.
2. Moisture: Determined by weight loss during drying under specified conditions.
3. Protein: In addition to true protein nitrogen this includes all nitrogen contributed by free amino acids, peptides, and certain other nitrogen containing compounds.
4. Carbohydrates: Computed as the difference between 100 and the sum of the percentages of moisture, protein, ether extract, and ash. This included crude fiber, starch, dextrin, sugar, and other undetermined solids.
5. Nitrogen free extract: Total carbohydrate less the crude fiber. This is considered a measure of the available carbohydrate.
6. Ether extract: Includes not only true fats but also other ether-soluble substances such as fatty acids, waxes, and plant pigments.

All the above determinations were made in accord with standard procedures of the Association of Official Agricultural Chemists (1).

In addition to the above, determinations were made of the provitamin A and ascorbic acid contents of the browse plants. These two vitamins were chosen because of their known importance to successful reproduction in ungulate mammals (9).

The vitamin analyses were made on fresh plant material representing 12 species at various levels of palatability to moose. Analyses were begun in March when the plants were still dormant and were continued to the end of the active growing period in mid-summer.

Carotene was extracted by the method of Moore and Ely (13) and was assayed by comparison with an Eastman Kodak Company preparation of carotene containing 10% α-carotene and 90% β-carotene.

Ascorbic acid was measured as total dehydroascorbic acid according to a modification of the Roe and Oesterling technique described by the association of vitamin chemists (2). The dinitrophenylhydrazine reagent used in this method measures dehydroascorbic acid. The ascorbic acid is oxidized to dehydroascorbic acid by the addition of norit to the metaphosphoric acid extract. Baird and Lane (3), in a study of herbaceous plants, found that "free" dehydroascorbic acid formed 10 to 25% of the "total" ascorbic acid and that while the "total" dehydroascorbic acid (ascorbic acid) values showed wide seasonal fluctuations the "free" dehydroascorbic acid changed only slightly.

Effect of Freezing Upon Vitamin Content

To examine the influence of the treatment of our samples upon the carotene and ascorbic acid contents, samples of *Pinus contorta* and of *Betula papyrifera* were kept in storage for a period of nine weeks. Analyses were made at weekly intervals.

On a basis of dry weight the carotene value in *Pinus* fell from 9.12 to 8.17 and in *Betula* from 34.57 to 30.85.

Ascorbic acid values declined, during the same period, from 165.28 to 151.32 for *Pinus* and from 230.13 to approximately 217.

These changes are not of sufficient degree to materially alter the conclusions reached as a result of our analyses.

Results

Floral Composition

In the area under study the climax plant association is dominated by spruce, sometimes in association with alpine fir. Douglas fir and lodgepole pine appear as pioneers in an early stage and remain in the association through the life span of the successful trees; they are not, however, characteristic of the climax in its completed state.

From Table I it can be seen that in Area 1 few conifers have become established and that they are all in the smallest size classes. In this area a total of 75 trees occurred on the sample quadrats.

In Area 2, on quadrats of similar area, on the other hand, there was a great increase in the total number of trees present to 805 of which more than half are lodepole pines below 54 in. in height. Twenty-five Douglas fir, 23 of them in the two smallest size classes, had also become established.

The counts for Area 3 reveal the emergence of the spruce as the dominant species. All age classes are represented for this tree, even though the largest number are in the 18 in. D.B.H. class. The Douglas fir and lodgepole pine are not reproducing in this area and almost all individuals are in the larger size classes.

TABLE I

RELATIVE CONIFER DENSITY AT THREE STAGES OF SUCCESSION IN THE SPRUCE-BALSAM ZONE, AREAS 1, 2, AND 3

Species	Size classes						
	Trees less than 54 in.		Diameter at breast height of trees over 54 in.				
	18 in. high or less	18 – 54 in. high	0.1–1 in.	1 – 2 in.	2 – 4 in.	4 – 8 in.	8 – 18 in.
Area 1							
Spruce (*Picea Engelmanni*)	36	9					
Douglas fir (*Pseudotsuga taxifolia*)							
Lodgepole pine (*Pinus contorta* var. *latifolia*)	9	19	2				
Area 2							
Spruce	41	16	9	16	13	2	
Douglas fir	14	9	2				
Lodgepole pine	394	166	22	18	25	54	4
Area 3							
Spruce	3	7	4	5	9	11	28
Douglas fir	1			2	7	17	9
Lodgepole pine			1		2	16	17
Alpine fir (*Abies lasiocarpa*)		2					

In this group of trees, alpine fir is the most palatable and least abundant. Lodgepole pine is eaten occasionally and Douglas fir and spruce virtually not at all.

It will be apparent, therefore, that the lodgepole pine, inasmuch as it is readily available in Areas 1 and 2, offers a potential food source, whereas in Area 3, the small numbers of alpine fir offer almost the only palatable browse contribution by the conifers. The spruce alone is accessible in Area 3, and it is not eaten.

Much the same situation exists with respect to the deciduous trees and shrubs. The palatable species of the earliest seral stages of forest succession are "upland" willow, aspen, paper birch, serviceberry, red osier dogwood, hazel, and high-bush cranberry. The unpalatable species, or those of such short growth as to be unavailable during the winter snow period, are rose, buffalo berry, thimbleberry, ocean spray, snowberry, black twinberry, Oregon grape, blueberry, and juniper.

Table II illustrates the situation on the three study areas. The total ground cover contributed by the palatable species within reach of moose is in Area 1, 19.5%; in Area 2, 10.9%, and in Area 3, 6.4%; thus demonstrating a threefold decrease in the density of available, palatable, deciduous browse accompanying the ageing of the forest.

At the same time the unpalatable or unavailable species demonstrate an increase in density of almost the same proportions as the decrease in palatable species.

TABLE II

PER CENT GROUND COVER PROVIDED BY SHRUBS AND DECIDUOUS TREES AVAILABLE TO MOOSE

	Area 1	Area 2	Area 3
Palatable species (winter)			
Upland willow (*Salix Scouleriana* and *S. Bebbiana*)	3.1*	.74	1.59
Paper birch (*Betula papyrifera*)	.13	1.15	.10
Serviceberry (*Amelanchier florida*)	4.03	4.39	3.66
Aspen (*Populus tremuloides*)	11.35	4.43	.10
Red osier dogwood (*Cornus stolonifera*)	0.04	—	Trace
Hazel (*Corylus californica*)	0.78	.20	.58
High-bush cranberry (*Viburnum pauciflorum*)	Trace	—	.37
Per cent palatable	19.5	10.9	6.4
Unpalatable species and those unavailable in winter			
Rose (*Rosa* sp.)	6.94	3.18	11.79
Buffalo berry (*Shepherdia canadensis*)	1.67	.66	4.57
Thimbleberry (*Rubus parviflorus*)	.58	Trace	.32
Ocean spray (*Spiraea discolor*)	.49	3.92	2.75
Snowberry (*Symphoricarpus racemosa*)	.04	—	.25
Black twinberry (*Lonicera involucratum*)	—	—	1.09
Oregon grape (*Berberis nervosa*)	.45	.49	2.45
Blueberry (*Vaccinium* spp.)	.86	3.30	4.21
Juniper (*Juniperus communis*)	—	1.34	1.49
Per cent unpalatable	11.0	12.9	28.9
Total per cent coverage	30.5	23.8	35.3

* *All percentages expressed in relation to total ground area in the quadrats.*

In summary, then, aspen and willow dominate the flora of six-year-old Area 1 while on Area 2 lodgepole pine, and on Area 3 spruce, have succeeded to dominance. The combined influence of these floral changes, with forest ageing, viz. decreased availability of the moderately palatable lodgepole pine, dominance of the unpalatable spruce, reduction in density of the highly palatable deciduous trees and shrubs, and increased density of those unpalatable or unavailable, result in a pronounced reduction in the quantity of moose food produced by the forest. This is the first important aspect of the degeneration of the carrying capacity of a forest in the Taiga formation as it approaches its climax.

Carotene

Carotene determinations were made on the six important browse species found in common upon all three of the experimental areas. One additional species, *Populus trichocarpa* (the black poplar), was found upon Area 1 only. For purpose of comparison with the situation in the deciduous plants favored as food by moose, three species of coniferous trees: Engelmann spruce (*Picea*

Engelmanni), not eaten at all by moose, Douglas fir (*Pseudotsuga taxifolia*), seldom eaten, and lodgepole pine (*Pinus contorta*), occasionally eaten in fair amounts were collected upon a site similar to Area 2 and analyzed.

The results of the carotene determinations are shown in Tables III and IV. The values have been expressed as milligrams per 100 gm. for both wet and dry weight. Twig samples collected in March, late in the period of winter dormancy, gave uniformly low values, the majority being below 2 mgm. per

TABLE III

CAROTENE CONTENT OF DECIDUOUS TREES AND SHRUBS PALATABLE TO MOOSE

Date	Area 1			Area 2			Area 3		
	Weeks stored	Mgm./100 gm.		Weeks stored	Mgm./100 gm.		Weeks stored	Mgm./100 gm.	
		Wet	Dry		Wet	Dry		Wet	Dry
Betula papyrifera (Paper birch)									
March 28	9	0.55	0.76	8	0.49	0.72	7	0.24	0.36
June 5	3	4.48	17.73	4	5.90	27.61	1	5.38	26.87
June 29	4	5.86	14.58	4	9.45	33.32	5	8.06	25.18
July 19	2	4.82	19.01	1	6.56	27.74	3	5.21	27.19
Amelanchier florida (Service-berry)									
March 28	9	0.25	0.41	8	2.65	3.66	8	0.304	0.463
June 5	2	3.65	9.74	4	1.95	8.68	3	4.84	22.37
June 29	4	7.18	16.13	4	7.99	20.44	5	12.90	39.41
July 19	2	3.43	8.85	1	7.05	19.20	3	8.50	26.76
Cornus stolonifera (Red osier dogwood)									
March 28	9	0.098	0.13	8	2.31	3.41	9	0.45	0.65
June 5	2	3.90	13.09	4	4.29	18.17	2	6.58	23.86
June 29	4	7.50	18.30	4	10.77	29.46	5	14.55	20.18
July 19	2	4.93	14.74	3	9.40	29.77	1	7.23	26.69
Salix Scouleriana and *S. Bebbiana* (Upland willow)									
March 28	9	0.71	1.22	8	0.70	1.12	8	0.54	0.82
June 5	2	2.70	9.94	4	3.34	13.67	2	7.95	29.63
June 29	4	3.90	10.14	4	8.33	22.65	4	9.69	28.53
July 19	1	4.00	10.51	3	6.57	20.79	2	5.95	18.39
Populus tremuloides (Aspen)									
March 28	9	0.42	0.74	8	1.08	1.61	8	0.315	0.503
June 5	3	3.63	14.64	3	4.03	17.14	3	1.78	8.98
June 29	4	8.54	19.39	4	8.31	21.34	4	3.63	19.28
July 19	2	4.69	11.46	1	9.30	25.17	3	3.375	12.14
Corylus californica (Hazel)									
March 28	9	0.30	0.43	9	2.21	3.14	9	0.385	0.601
June 5	3	2.53	8.96	3	7.30	20.98	2	5.85	25.14
June 29	4	7.10	15.21	4	7.48	18.57	5	16.69	40.48
July 19	1	3.68	9.68	2	7.48	21.79	3	8.175	25.22

NOTE: *Percentage moisture of the tissue may be readily obtained from the formula,*

$$\%\ moisture = 100 - \frac{100 \times carotene\ content\ wet}{carotene\ content\ dry}.$$

TABLE IV

CAROTENE AND ASCORBIC ACID IN CONIFEROUS TREES

Date	Weeks stored	Carotene		Ascorbic acid	
		Mgm. per 100 gm.		Mgm. per 100 gm.	
		Wet	Dry	Wet	Dry
Pinus contorta var. *latifolia* (Lodgepole pine)					
March 28	9	3.44	6.78	236.75	467.14
June 5	2	3.03	7.57	34.88	87.15
June 29	3	3.49	9.90	43.83	124.26
July 19	2	2.69	7.33	96.13	321.61
Pseudotsuga taxifolia (Douglas fir)					
March 28	8	1.33	2.58	282.20	548.28
June 5	2	1.95	4.86	93.65	233.42
June 29	3	4.48	7.71	82.63	142.57
July 19	2	2.50	6.19	123.67	306.42
Picea Engelmanni (Spruce)					
March 28	9	2.53	3.08	271.50	330.05
June 5	1	2.60	6.63	86.23	220.03
June 29	3	2.72	7.68	68.03	192.06
July 19	2	2.69	7.33	79.07	215.39

NOTE: *Percentage moisture in wet tissues may be obtained from formula given in Table III. "Ascorbic acid" values are for "total" dehydroascorbic acid ("free" dehydroascorbic plus oxidized ascorbic acid).*

100 gm. However, the values for serviceberry (*Amelanchier*), red osier dogwood (*Cornus*), and hazel (*Corylus*) in Area 2 reached 3.66, 3.41, and 3.14 mgm. per 100 gm. dry weight respectively.

A low carotene content in winter browse is to be expected when it is considered that the twigs contain little or no chlorophyll. Zscheile, Beadle, and Kraybill (15) found that the ratio of chlorophyll to carotene is fairly constant. We should then see a rise in carotene values as the season progresses and this will be demonstrated.

In this regard it is pertinent to note that the "evergreen" coniferous trees showed very small seasonal variation in carotene content and had carotene values during the winter resting period of from one-half to one-third of the summer values (*Pseudotsuga* and *Picea*) to two-thirds the summer value in *Pinus contorta* (Table IV). These species then, even though they have winter carotene values insignificant in comparison with those of the deciduous vegetation in summer, appear as winter sources of substantially greater amounts of this vitamin than the amount available in the winter deciduous vegetation.

By June 5, when the second series of samples was taken, spring growth was fairly well advanced. The majority of samples show a definite carotene increase. In 73% of the samples carotene had increased to more than 10 times the low winter content. The average value for carotene in deciduous

species in all areas was then 15.43 mgm. per 100 gm., with a low of 8.68 mgm. for *Amelanchier* and a high of 29.63 for upland willow (*Salix Scouleriana* and *Bebbiana*).

The June 5 samples had small leaves which were less than half the mature size and the moisture content was about twice that of the twig samples of Mar. 28.

The samples of June 29 represented the browse at the peak of summer luxuriance. The leaves were near maturity and the moisture content about the same as that of the June 5 sample. Of the samples assayed 64% were found to have higher carotene content than corresponding samples taken early in the month. The highest content was that of *Corylus* on Area 3 with 40.48 mgm. per 100 gm. and the lowest, *Salix* in Area 1 with just 10.14 mgm. per 100 gm. The average value for the June 29 samples on all areas was 20.39 mgm. per 100 gm.

The July 19 samples represent browse after it has passed the peak of maturity. The summer season is beginning to wane and vegetative conditions are stabilizing. Of the samples assayed, 33% had higher carotene values than their previous counterparts while in the remaining 67% the values were virtually unchanged or substantially lower. The average for the July 19 samples was 20.31 mgm. per 100 gm., and thus almost identical with the June 29 condition.

Despite this similarity in mean value of all samples taken on June 29 and July 19 there seems to be convincing evidence that the seasonal peak in carotene values on the part of most of the browse species is reached toward the end of June and is followed by a gradual decline, presumably to winter levels. A seasonal fluctuation even during the growing period has been demonstrated by other workers (10) who found a consistent seasonal change in carotene content subject to climatic influence that could change the time of appearance of maxima. Hoar, Barberie, and Davidson (12) working with wild herbaceous plants found that maximum content is reached in July with a steady decline thereafter. Other recent papers record similar findings in different groups of plants (14 and 15).

Table V has been prepared to facilitate the comparison of the plants in terms of carotene value, during the course of their succession toward the climax. The values given are means for the entire period of sampling. Aspen (*Populus tremuloides*) and *Betula papyrifera* reached their maximum values on Area 2 but the significance of this is not understood. All the other species had a mean carotene value steadily increasing from Area 1 to Area 3. In the species of plants under examination it would appear that the carotene production increased with maturity and that this effect persisted even under the less favorable growing conditions obtaining in Area 3, where the coniferous species have achieved dominance. It is concluded then that the higher values found to hold in Area 3 are explainable on the basis of the maturity of the individual plants contributing to the sample.

TABLE V

COMBINED AVERAGES OF CAROTENE IN THE EXPERIMENTAL AREAS DURING SPRING AND SUMMER

Species	Carotene in mgm./100 gm. dry weight		
	Area 1	Area 2	Area 3
Betula papyrifera	13.02	22.35	19.90
Amelanchier florida	8.78	12.99	21.75
Cornus stolonifera	11.56	20.20	22.85
Salix Scouleriana and *S. Bebbiana* (Upland willow)	7.95	14.56	19.34
Populus tremuloides	11.56	16.57	10.22
Corylus californica	8.57	16.12	22.87

Ascorbic Acid

The ascorbic acid values are shown in Tables IV and VI. As was the case with carotene, the ascorbic acid content of deciduous vegetation is at a low during the dormant period and increases as the chlorophyll content of the plant material increases.

In general the same seasonal trends appear in ascorbic acid as in carotene content of the deciduous vegetation although there is a tendency for maximum values to be reached somewhat later in the season. Thus only 37% of the analyses showed maxima on June 29 while 54% showed them on July 19. Inasmuch as sampling was discontinued on that date it is not known whether the peak in all species had been reached.

In the group of six browse plants present on all three areas *Populus tremuloides* appears as the most important source of ascorbic acid during the summer season. It is followed by *Betula papyrifera*, *Corylus*, *Cornus stolonifera*, *Amelanchier florida*, and *Salix* in descending order.

From the standpoint of the best sources of this vitamin in the winter diet, upland willow and *Amelanchier* are the most important species.

The ascorbic acid values of the coniferous trees follow a pattern distinctively different from that of any of the deciduous species. The values determined for *Pinus contorta*, *Pseudotsuga taxifolia*, and *Picea Engelmanni* are given in Table IV. In each instance these trees show their maximum values in March and decline thereafter. In all three species these maxima compare very favorably with mid-summer values for the deciduous species. It is regrettable that it was not possible to carry the analyses of these species through the entire year and to determine whether the March values are truly representative of the dormant period. If they are, the palatable members of this group of trees constitute a most important source of ascorbic acid during the winter months when the values in deciduous plants are low.

In order to examine the relative position of the three areas with respect to the ascorbic acid content of the vegetation produced on them, two tables of values have been prepared. These show the analyses for the dormant period

TABLE VI

ASCORBIC ACID CONTENT OF DECIDUOUS TREES AND SHRUBS PALATABLE TO MOOSE

Date	Area 1			Area 2			Area 3		
	Weeks stored	Mgm./100 gm.		Weeks stored	Mgm./100 gm.		Weeks stored	Mgm./100 gm.	
		Wet	Dry		Wet	Dry		Wet	Dry
Betula papyrifera (Paper birch)									
March 28	9	45.38	62.40	8	24.17	35.69	7	22.38	33.52
June 5	3	77.88	308.19	4	104.18	487.50	1	90.38	451.44
June 29	4	209.63	521.73	4	209.63	739.17	5	83.33	260.32
July 19	2	187.23	738.29	1	135.50	572.69	3	92.80	484.60
Amelanchier florida (Service-berry)									
March 28	9	45.63	74.28	8	42.77	59.08	8	36.14	55.01
June 5	2	88.92	237.37	4	43.40	193.40	3	24.10	111.37
June 29	4	129.75	291.44	4	141.38	361.77	5	103.08	314.94
July 19	2	196.33	506.53	1	113.40	308.82	3	76.83	241.91
Cornus stolonifera (Red osier dogwood)									
March 28	9	38.66	51.78	8	59.73	88.18	9	32.84	47.61
June 5	2	90.23	302.99	4	58.67	248.50	2	54.22	196.59
June 29	4	158.60	268.72	4	171.43	270.22	5	127.55	352.17
July 19	2	178.43	533.90	3	136.17	431.19	1	107.30	396.09
Salix Scouleriana and *S. Bebbiana* (Upland willow)									
March 28	9	49.63	85.45	8	41.35	65.90	8	41.12	62.59
June 5	2	59.02	217.30	4	90.83	371.64	2	57.25	285.39
June 29	4	158.60	412.16	4	113.92	309.82	4	99.90	294.08
July 19	1	103.13	270.90	3	88.13	278.89	2	82.47	254.85
Populus tremuloides (Aspen)									
March 28	9	39.48	69.49	8	28.49	42.59	8	27.03	43.15
June 5	3	130.58	526.53	3	113.23	481.42	3	91.67	462.51
June 29	4	208.13	472.48	4	295.00	757.58	4	138.83	734.62
July 19	2	337.50	824.98	1	306.50	829.50	3	162.43	584.49
Corylus californica (Hazel)									
March 28	9	20.13	28.97	9	34.17	48.55	9	25.10	39.20
June 5	3	72.15	255.58	3	73.55	312.18	2	54.72	235.15
June 29	4	267.50	573.05	4	189.45	470.33	5	143.83	348.93
July 19	1	217.17	571.20	2	196.33	571.89	3	83.83	258.58

NOTE: *See footnote Table IV.*

as represented by the March samples (Table VII) and the highest values reached during the summer season (Table VIII).

Except for the two striking inconsistencies that we cannot explain, namely the exceedingly low value for *Corylus* on Area 1 and the very high value for *Cornus* on Area 2, the winter analyses reveal a consistent downward trend during the progress of forest succession. With the exceptions mentioned, the youngest area (1) has the highest winter values and the oldest area (3) the lowest.

TABLE VII

ASCORBIC ACID CONTENT OF BROWSE PLANTS DURING THE WINTER PERIOD*

Species	Area 1	Area 2	Area 3
Betula papyrifera	62.40	35.69	33.52
Amelanchier florida	74.28	59.08	55.01
Cornus stolonifera	51.78	88.18	47.61
Salix Scouleriana and *S. Bebbiana*	85.45	65.90	62.59
Populus tremuloides	69.49	42.59	43.15
Corylus californica	28.97	48.55	39.20

* *Expressed as mgm./100 gm. dry weight in the March sample.*

TABLE VIII

SUMMER MAXIMA IN ASCORBIC ACID CONTENT* OF DECIDUOUS TREES AND SHRUBS PALATABLE TO MOOSE

Species	Area 1	Area 2	Area 3
Betula papyrifera	738.29	739.17	484.60
Amelanchier florida	506.53	361.77	314.94
Cornus stolonifera	533.90	431.19	396.09
Salix Scouleriana and *S. Bebbiana*	412.16	371.64	294.08
Populus tremuloides	824.98	829.50	734.62
Corylus californica	573.05	571.89	348.93

* *Expressed as mgm./100 gm. dry weight.*

The same trend is apparent also in the examination of the maximum summer values in Table VIII. Although there is no appreciable difference in the values on Area 1 and 2 with respect to *Betula*, *Populus*, and *Corylus* there is in every instance a marked decline in maximum values on Area 3, and in *Amelanchier*, *Cornus*, and *Salix* the trend is evenly downward from Area 1 through 2 to 3.

It can be adduced then that the young forest areas, the earliest shrub stages of forest succession, provide diets both winter and summer with ascorbic acid values higher than those in more advanced stages of forest succession.

Nutrients Other Than Vitamins

The nutritive values of 17 species of plants available as winter food for moose in the Quesnel area are given in Table IX. This table concerns a wider variety of species than occurred on the three areas studied for the details of forest succession. The analyses of lodgepole pine, Douglas fir, spruce, aspen, upland willow, paper birch, dogwood, serviceberry, and hazel were made upon samples obtained upon the experimental areas. The remaining species were growing in the same general region, but not actually on our plots. Analyses refer to the period between November and May, with samples taken for

TABLE IX

PERCENTAGE COMPOSITION OF MOOSE FOODS

Common name	Scientific name	No. of samples	Moisture	Protein	Ether extract	N-free extract	Mineral	Crude fiber
Alpine fir	*Abies lasiocarpa*	1	43.43	6.39	12.51	58.41	3.53	19.16
Lodgepole pine	*Pinus contorta*	13	54.30	6.90	8.47	57.23	2.42	24.98
Douglas fir	*Pseudotsuga taxifolia*	13	50.27	6.53	7.83	62.74	3.22	19.91
Spruce	*Picea Engelmanni*	13	47.51	5.40	6.59	60.75	4.01	21.58
Aspen	*Populus tremuloides*	13	46.34	7.10	7.71	52.95	4.16	28.07
Black poplar	*Populus trichocarpa*	7	48.21	6.08	15.26	51.17	3.42	24.07
Mountain maple	*Acer glabra*	1	45.31	5.91	2.42	54.15	4.20	33.32
Swamp willow	*Salix* species*	4	49.77	6.32	5.31	61.92	2.46	23.99
Upland willow	*Salix* species**	14	46.73	5.91	4.12	55.18	4.35	31.86
Paper birch	*Betula papyrifera*	14	43.75	6.98	8.39	52.01	2.85	29.78
Bog birch	*Betula glandulosa*	6	34.99	6.10	8.23	56.40	2.10	27.17
Red osier dogwood	*Cornus stolonifera*	13	46.66	4.84	4.89	57.53	4.00	28.75
Serviceberry	*Amelanchier florida*	13	42.34	5.45	3.17	58.52	4.39	28.52
Hazel	*Corylus californica*	13	45.36	6.58	1.95	59.13	5.77	26.75
Buffalo berry	*Shepherdia canadensis*	1	38.52	14.66	1.98	58.92	3.10	21.34
Beard moss	*Usnea barbata*	1	8.54	2.41	7.31	86.20	1.70	22.38
Alder	*Alnus sitchensis*	6	46.77	9.95	6.57	57.04	2.77	23.68
Aspen (bark)	*Populus tremuloides*			12.66	14.21	43.07	5.81	24.24

* *Salix McCalliana, S. mertillifolia, S. pedicellaris.*
** *Salix Scouleriana, S. Bebbiana.*

analyses in November, late December, early January (one area only), March, and May. Analyses were, in each case, confined to the current season's growth.

Comparison of these values with those obtained by Hellmers is possible for several closely related species present in both series. This comparison is made in Table X and reveals a fairly close agreement between the plants of the

TABLE X

COMPARISON OF NUTRITIVE VALUES OF WOODY PLANTS IN B.C. AND PENNSYLVANIA

Species	Protein		Ether extract		Nitrogen-free extract		Crude fiber		Mineral matter	
	B.C.	Penn.	B.C.	Penn.	B.C.	Penn.	B.C.	Penn.	B.C.	Penn.
Aspen	7.10	7.9	7.71	9.8	52.95	51.1	28.07	28.2	4.16	3.0
Hazel	6.58	6.1	1.95	2.8	59.13	53.4	26.75	34.2	5.77	3.6
Dogwood	4.84	4.7	4.89	4.5	57.53	56.6	28.75	31.5	4.00	2.7
Prairie (upland) willow	5.91	7.5	4.12	4.7	55.18	50.1	31.86	35.0	4.35	2.9

two regions. The samples from the Quesnel region of British Columbia are fairly consistently higher in protein, mineral, and nitrogen-free extracts and lower in crude fiber content than are those from Pennsylvania. These are all qualities that should make them better game foods. On the other hand, the Pennsylvania samples are generally higher in ether extractives.

Clarke and Tisdale (4) give protein values in October for such broadleaved range plants as *Artemisia frigida*, *Artemisia cana*, *Atriplex Nuttalli*, and *Eurotia lanata*, all of them forage plants of the Canadian prairies. All possess values well in excess of those determined for the plants used by moose.

Davenport (5) gives the results of a series analyses of trees and shrubs used by deer in Michigan. The protein values all ranged between 5.20% and 9.79% with only three of 15 species below 7%. These then are considerably higher in protein content than is the browse available to moose in the Quesnel area. Deer feeding on the Michigan food plants still lost weight steadily during the winter.

A general ranking of the 17 species of plants analyzed during this study in order of their approximate value as foods for ungulates is of value in the appraisal of ranges and in the planning of range management. Rather than attempt to seriate them we have separated them into three major groups as follows:

Highest quality: alpine fir, lodgepole pine, Douglas fir, aspen, paper birch, buffalo berry,* alder.

Intermediate quality: spruce,* black poplar, swamp willow, upland willow, bog birch, hazel.

Lowest quality: mountain maple, dogwood, serviceberry, beard moss.

Of the 10 species graded by us as of high or intermediate quality, alpine fir, aspen, paper birch, bog birch, upland willow, and hazel are palatable and used fairly extensively by moose. On the other hand, two of the most palatable species, dogwood and serviceberry fall into the deficient category.

Only one species, *Shepherdia*, has a protein value in excess of 10%, while 10 of the 17 have values between 6 and 10% and only three exceed 8%. Fraps and Cory (7) have attempted an appraisal of the desirable protein levels for range animals (cattle, sheep, and goats) and arrived at the conclusions indicated in Table XI.

TABLE XI

GRADES FOR PROTEIN IN FORAGE FOR RANGE ANIMALS

Grade	Interpretation	Crude protein
1	High	15.00 +
2	Good	10.50 – 14.99
3	Fair	6.00 – 10.49
4	Deficient	3.00 – 5.99
5	Very deficient	0 – 2.99

On this basis only *Shepherdia* can be classified as good and this is not eaten by moose.

* *Unpalatable to moose.*

Davenport (5) sets a higher protein level for a satisfactory diet and states that for the feeding of domestic livestock a 12% protein diet is considered the minimum for a maintenance ration. On this basis all the browse species consumed by moose during the winter would be rated as deficient. The strong possibility emerges that moose are adapted to living upon lower quality nutrients than are domestic ungulates. The examination of this possibility would be a valuable contribution toward a better interpretation of range capacity.

Relative Quality of Wood of Different Ages

In an overstocked moose range, such as much of central British Columbia, food shortage during the late winter forces the moose to browse repeatedly upon twigs from which the more succulent tips had been removed earlier. To obtain a measure of the concomitants of this situation with respect to the nutrients available to moose, a small series of analyses were undertaken.

The hazel and upland willow were chosen as representative of the shrubs frequently subjected to this type of use and the results of the analyses are presented in Table XII. Hazel samples were taken in March; those of upland willow in February.

TABLE XII

CHEMICAL ANALYSIS OF TWIG GROWTH, ONE, TWO AND THREE YEARS OLD

Years of growth	% moisture	Crude protein	Ether extract	N-free extract	Crude fiber	Mineral
Hazel						
1	43.98	7.96	2.13	60.02	23.25	5.64
2	38.41	5.12	2.12	56.12	31.75	4.89
3	40.07	4.11	1.26	50.01	41.62	3.82
Willow						
1	43.10	7.22	4.32	54.10	31.73	2.63
2	43.10	5.69	2.70	50.20	38.72	2.69

The decline in values in the parts of the twig older than the current season's growth is large and consistent. It serves to elucidate yet another factor contributing to the rapid onset of malnutrition in animals forced to exist upon overstocked ranges.

Loss of Quality Through the Dormant Period

Several earlier studies have shown that even during the quiescent period certain plants reveal changes in the content of nutrients. Sometimes these were of a variable nature while at other times they showed regular trends. Few of these studies have applied to native trees and shrubs growing under the rigorous climatic circumstances encountered on the present study area.

Hellmers (11) reported upon the monthly changes in nutritives in a series of eight woody plants used as winter food by deer in Pennsylvania. His findings indicated a fluctuating pattern resulting in a small decline in protein, nitrogen-free extract, and mineral content between November and April, accompanied by a gradual rise in crude fiber during the same period. In terms of the reduction of protein content during the winter dormant period Helmers' figures show only a mean 0.6% decline between the November analyses and the April analyses, approximately 11% reduction on the larger figure.

Einarsen's (6) studies of deer food plants in coastal Oregon suggest a much greater decline. Here mean protein value for six shrub and tree species in November was 9.37% while in April it was only 7.03%—a decline of 25%. These values were taken from an area of unusually high nutritive values.

The dormant period in the present study area extended from late October to May and, in consideration of the demonstrated seasonal trend in certain nutrients, samples were taken for analysis at approximately bimonthly intervals from November to May.

The analyses of nine species of plants common to all three experimental areas and arranged to illustrate the values at bimonthly intervals during the dormant period are given in Table XIII. The January figures are, in general, based upon one sample only; while the other three months are represented by at least four samples in each case. In general, six of the nine species show a slight reduction in protein content during the progress of the winter, just four of the nine showed losses in ether extract, five showed increases in crude fiber content, and three revealed upward trends in nitrogen-free extract. Mineral matter showed the most pronounced trend with all the deciduous species increasing in this respect through the winter months. The six species that underwent a loss in protein lost a mean 0.63% in value or about 9% of the November amount down to the lowest point, in March. This amount is not very different from the 11% loss reported by Hellmers (11) from Pennsylvania but is sharply divergent from the 25% loss demonstrated by Einarsen and believed by him to result from lack of sunlight. It is suggested that the very great loss of quality demonstrated in his studies on the Oregon coast may be peculiar to the rain forest area of the Pacific Coast where the winter can be almost sunless.

Changes Accompanying Forest Succession

In Table XIV the results of the analyses of each species are expressed in each instance as the mean of all the samples taken during the winter. These means are arranged with respect to the areas from which they were taken. It should be recalled that Area 1 is the youngest, and bears a plant association just six years from denudation. Area 2 bears a young forest about 20 years old and Area 3 has the climax coniferous association well established. It is about 70 years old.

TABLE XIII

SEASONAL VARIATION IN CHEMICAL COMPOSITION OF AVAILABLE BROWSE PLANTS EXPRESSED AS A MEAN OF ALL SAMPLES

	Spruce	Douglas fir	Lodge-pole pine	Aspen	Paper birch	Upland willow	Hazel	Dog-wood	Service-berry
Protein									
November	5.73	6.85	7.00	7.20	7.24	6.02	7.27	4.73	5.33
January	5.47	6.14	7.26	6.83	7.00	6.08	6.87	4.94	5.86
March	5.20	6.24	6.71	6.94	6.44	5.79	5.92	4.81	5.46
May	5.26	6.10	6.91	7.24	7.26	5.93	6.47	4.94	5.47
Ether extract									
November	6.53	8.24	6.98	7.98	9.19	4.48	2.14	4.94	3.51
January	6.70	8.05	9.57	6.95	8.50	3.74	1.60	4.95	2.94
March	7.17	8.13	9.06	8.02	7.18	4.11	1.75	4.93	2.87
May	6.06	7.07	9.10	7.33	8.48	3.76	2.05	4.78	3.19
Nitrogen-free extract									
November	59.75	62.19	57.59	50.42	51.09	55.14	59.73	57.24	58.95
January	59.53	60.40	55.20	54.88	51.36	53.32	61.09	57.92	57.25
March	59.55	61.68	56.54	53.94	52.66	55.24	58.37	58.06	58.89
May	63.26	64.95	58.06	54.04	52.05	55.34	58.80	57.19	58.53
Crude fiber									
November	24.29	19.77	25.08	29.77	29.77	30.01	25.55	29.29	28.68
January	23.89	20.03	25.10	29.92	29.92	32.07	24.45	28.39	29.35
March	23.70	20.45	25.19	31.03	31.03	30.35	28.28	28.08	28.43
May	21.65	18.74	23.66	28.84	28.84	30.20	26.42	28.97	28.05
Minerals									
November	3.75	2.97	2.36	3.69	2.74	4.42	5.31	3.80	4.03
January	4.41	3.38	2.87	3.92	2.83	3.47	5.99	3.80	4.60
March	4.40	3.51	2.52	4.33	2.70	4.51	5.69	4.11	4.33
May	3.79	2.97	2.27	4.52	3.11	4.78	6.26	4.12	4.59
Moisture									
November	49.15	51.83	54.92	48.75	42.82	46.01	45.40	47.27	42.35
January				48.78	41.03	45.56	55.27	46.37	44.11
March	46.76	46.94	54.86	44.89	40.93	45.55	44.74	43.81	42.36
May	45.19	59.76	52.48	46.37	49.33	47.07	45.98	48.97	41.86

TABLE XIV

CHANGES IN CHEMICAL COMPOSITION ACCOMPANYING FOREST AGEING

Area	Spruce	Douglas fir	Lodge-pole pine	Aspen	Paper birch	Upland willow	Hazel	Dog-wood	Service-berry
Mean winter values for crude protein									
Area 1	5.68	6.71	7.10	6.47	7.05	5.83	7.06	4.70	5.60
Area 2	4.97	6.45	6.79	7.05	6.88	5.32	6.53	4.70	5.21
Area 3	5.12	5.44	6.55	7.53	6.63	5.91	5.89	4.72	5.37
Change	−10%	−19%	−6%	+15%	−6%	+1%	−17%	0	−5%
Mean winter values for ether extract									
Area 1	7.95	8.29	9.43	8.62	8.95	4.82	2.03	4.72	3.02
Area 2	6.47	7.99	8.49	7.83	8.13	3.66	1.92	4.81	3.02
Area 3	5.47	6.98	6.66	6.04	6.96	3.73	1.85	5.16	3.53
Change	−31%	−16%	−29%	−30%	−22%	−23%	−9%	+9%	+17%
Mean winter values for nitrogen-free extract									
Area 1	59.96	63.00	57.92	53.41	52.20	56.04	60.27	56.72	59.45
Area 2	60.81	62.62	56.43	52.64	52.88	54.81	59.76	58.34	58.18
Area 3	61.32	63.03	58.40	52.32	51.25	54.89	55.86	56.95	58.37
Change	+2%	0	+1%	−2%	−2%	−2%	−7%	0	−2%
Mean winter values for crude fiber									
Area 1	22.20	18.92	23.21	27.75	29.14	31.30	24.45	30.33	28.02
Area 2	23.68	19.70	26.11	28.32	28.98	29.95	25.93	27.99	29.70
Area 3	24.27	21.38	26.16	29.30	32.22	38.06	30.75	29.15	27.99
Change	+9%	+13%	+13%	+6%	+10%	+21%	+26%	−4%	0
Mean winter mineral content									
Area 1	4.09	3.08	2.34	3.75	2.66	3.28	6.19	3.58	3.91
Area 2	4.07	3.25	2.17	4.50	3.13	4.90	5.83	4.17	3.92
Area 3	3.82	3.43	1.92	4.60	2.91	5.53	5.68	4.01	4.74
Change	−6%	+11%	−17%	+23%	+9%	+69%	−8%	+11%	+21%

In six out of the nine species studied protein values were highest in Area 1; in four of these they were lowest in Area 3. For each species the percentage increase or decrease in protein content accompanying ageing of the forest stand

has been calculated using the value in Area 1 as 100%. It will be noted that in the six species in which protein value decreased with ageing, the decrease varied from 5 to 19%.

Ether extractives showed a similar trend with percentile changes even greater than those of protein. In this category the loss in values in seven of the nine species varied from 9% to 31%.

Nitrogen-free extract showed no clear trends. Five of the nine species, on the basis of the present studies, actually reveal a decline in nitrogen-free extractives, but the picture is not convincing. It may be significant, however, that the three conifers showed slight positive trends while five of the six deciduous species gave indication of negative trends.

Crude fiber, on the other hand, increased substantially in all but two of the species. This suggests a decline in digestible material.

Total mineral content as revealed by ash gives a somewhat variable picture with five species showing substantial increases concomitant with ageing of the forest and three other species, decreases.

Summary and Discussion

This study has revealed that in the Taiga forest formation the sequence of floral changes leading toward the climax plant association dominated by spruce is accompanied by a great reduction in the quantity of browse available to the moose.

The analyses have demonstrated that the group of trees and shrubs palatable to and available to moose as food in the Quesnel area are of relatively uniform low nutritive quality. There are some notable exceptions to this as for example the high protein value of *Shepherdia* twigs and *Populus tremuloides* bark; the high ether-extractive values of *Abies*, *Populus trichocarpa*, and the bark of *P. tremuloides*; and the remarkably high carbohydrate rating of *Usnea*.

It is well known that the animal nutrients available from plants is at its lowest point during the period of dormancy. This has been found to be true for all the nutrients for which analysis has been made in the present study. A gradual decline in the content of protein, carbohydrates, and fats present in the dormant, woody, vegetation has been shown to take place here but to a less marked extent than in some other areas. The mean loss during dormancy in the species exhibiting this phenomenon was 9%.

The determinations of carotene content and ascorbic acid content indicate that the coniferous trees are the most concentrated source of carotene, while among the deciduous species the highly palatable upland willow leads all the others. Much the same is true of ascorbic acid. The content of this vitamin in coniferous twigs and foliage during the winter months is almost five times greater than that in upland willow, the most valuable source of this item among the deciduous species.

An investigation of the march of nutritional properties of vegetation in the three study areas representing successive stages in forest growth and succession has shown that the oldest areas bear vegetation with the highest carotene values and possibly mineral contents, but that in all other respects the youngest forest stage was, with few exceptions, producing the most nutritious food for the moose population. This change was most obvious in ether extractives and was somewhat less pronounced with respect to protein.

In general terms it can be concluded that the youngest stages in forest succession are, in this habitat, providing the most nutritious vegetation, but for certain nutrients access to older stands bearing well grown coniferous trees is desirable. It is also clearly evident that a flora containing several different palatable browse species is most important in the constitution of a satisfactory winter range.

Nutritive quality and palatability are not necessarily related. Thus *Shepherdia canadensis* and *Picea Engelmanni* are both of good quality, but unpalatable to moose in this area. Further, there is little to choose between the two types of willows analyzed. While one, the upland willow, is a preferred food the swamp willow is taken in reduced amounts.

Among the palatable species those of highest quality are *Pinus contorta*, *Populus tremuloides*, *Betula papyrifera*, and *Alnus sitchensis*. Those of intermediate quality are *Populus trichocarpa*, *Salix*, *Betula glandulosa*, and *Corylus californica;* while those lowest in nutrients are *Acer glabra*, *Cornus stolonifera*, and *Amelanchier florida*.

The results of this study, then, point to the desirability of winter range for browsing ungulates upon which there is a variety of palatable species predominantly in an early stage of growth, but with an intermixture of stands of other ages including areas bearing subclimax or climax associations including palatable coniferous species. The most desirable winter range for moose will be one well diversified as to species composition and age of stands, but predominantly of new growth following deforestation.

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SOME BIOCHEMICAL EFFECTS OF THIOURACIL ON THE RESPONSE OF THE IMMATURE PULLET TO ESTROGEN[1]

By R. H. Common,[2] T. J. Keefe,[3] and W. A. Maw[4]

Abstract

Thiouracil depressed the increases of serum calcium, serum riboflavin, and serum vitamin A evoked by estrogen in the sexually immature pullet. In these respects the effects of thiouracil resembled the effects of thyroxine. In contrast to thyroxine, however, thiouracil increased the hypertrophy of the liver and oviduct induced by estrogen. Estrogen did not significantly increase total liver riboflavin, whereas estrogen plus thiouracil produced a significant increase.

Introduction

Fleischmann and Fried (9) have shown that treatment with thyroxine depresses the response of the chick's serum calcium to treatment with estrogen, whereas the degree of hypertrophy of the oviduct induced by the estrogen is not appreciably affected by thyroxine. Others (6) have shown that thyroxine depresses the response of the immature fowl's serum riboflavin to estrogen, and at the same time depresses the increases in liver weight and weight of liver crude protein which are evoked by estrogen. Fleischmann and Fried (9) found that thiouracil increased serum cholesterol and that this increase was antagonized by thyroxine. Thiouracil did not affect the level of serum calcium and phosphorus. These workers offered the following interpretation of their observations:—(*a*) estrogen mobilizes cholesterol from the tissues to the plasma and thiouracil has the same effect; (*b*) thyroxine tends to keep cholesterol in the tissues and in this respect antagonizes both estrogen and thiouracil; (*c*) the effects of estrogen on serum calcium and phosphorus are secondary to the effects of estrogen on the synthesis of phospholipid and phosphoprotein in the liver. Fleischmann and Fried (9) concluded that the effects of estrogen on serum cholesterol, serum calcium, and phosphorus on the one hand, and on hypertrophy of the oviduct on the other hand, were separate and dissociable effects.

Since thyroxine reduces the response of both serum calcium and serum riboflavin to estrogen, it became of interest to examine the effects of thiouracil on the serum calcium and serum riboflavin of the estrogenized immature pullet. The present paper deals with experiments directed to this end.

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Experimental

General Technique and Analytical Methods

Care was taken to ensure that each of the pullets in any one experiment consumed the same total amount of food, a point of considerable importance in experiments of this nature in view of the stimulating effects of gonadal hormones on appetite (1).

The vitamin A contents of sera and livers were determined by the method of Dann and Evelyn (7). The calibration curve for the antimony trichloride reaction was checked against the Canadian Reference Solution of vitamin A acetate. The results are expressed as micrograms vitamin A (alcohol). Serum calcium was determined by Halverson's method as described by Peters and Van Slyke (13). Serum riboflavin was determined fluorimetrically by a macroadaptation of the method of Burch, Bessey, and Lowry (2). The micromethod described by these authors requires a microfluorimeter if the method is to be applied to mammalian sera or the sera of sexually inactive fowl. The amounts of riboflavin in the sera of estrogenized pullets are frequently sufficiently great, however, to allow of the procedure of Burch *et al.* (2) being applied on the macro scale using a Coleman photofluorometer (Model 12). Liver riboflavin was determined fluorimetrically by the procedure of Kodicek and Wang (11).

Experiment 1

Twenty cross-bred (New Hampshire ♂ × Barred Plymouth Rock ♀) pullets of the same strain and hatching were placed in an individual cage laying battery and assigned at random to five groups each of four pullets. The birds received a commercial growers' mixture.

The treatments of the five groups are indicated in Table I. Each bird of Groups 2, 3, 4, and 5 received six doses of 1.5 mgm. estradiol benzoate (Progynon B, Schering) administered by intramuscular injection on alternate mornings over the 12 day experimental period. Group 1 received injections of oily base only. Groups 3, 4, and 5 were given thiouracil by incorporation of the drug in their food at the levels indicated in Table I. The pullets were 82 days of age at the outset and 94 days of age at the end of the experiment. The results are summarized in Table I.

The pullets remained in excellent condition and consumed their daily food allowance regularly. No depressant effects of thiouracil on activity were observed. The variability of the data was such that relatively few of the differences between Groups 2, 3, 4 and 5 attained significance at the 5% point. However, those differences between Groups 1, 2, and 5 which attained significance justify the conclusion that thiouracil depressed the responses of serum riboflavin and serum vitamin A to the treatment with estrogen. Thiouracil, as was to be expected, increased thyroid weight to a significant degree as between Group 5 and either Group 1 or Group 2. The apparent tendency for estrogen to reduce thyroid weight (comparison of Groups 1 and 2) did not

TABLE I

EXPERIMENT 1. EFFECTS OF THIOURACIL ON CERTAIN BIOCHEMICAL RESPONSES OF THE IMMATURE PULLET TO ESTROGEN. AVERAGE RESULTS

	Group					L.S.D.*
	1	2	3	4	5	
	No. of pullets					
	4	4	4	4	4	
Total dosage estradiol benzoate, mgm.	Nil	6 × 1.5	6 × 1.5	6 × 1.5	6 × 1.5	
Thiouracil in food, %	Nil	Nil	0.05	0.10	0.20	
Live weight, initial, kgm.	0.87	0.93	0.89	0.84	0.87	
Live weight, final, kgm.	1.07	1.10	1.08	1.03	1.05	
Total food consumption, kgm.	0.83	0.83	0.83	0.83	0.83	
Ovary weight, gm.	0.30	0.22	0.25	0.20	0.23	N.S.**
Oviduct weight, gm.	0.08	7.4	8.4	6.0	6.7	2.4
Thyroid weight, mgm.	84	65	93	120	141	41
Liver weight, gm.	23.1	30.1	30.3	29.7	36.2	N.S.
Liver weight, gm./kgm. live weight	21.6	27.5	28.0	28.9	34.5	N.S.
Serum calcium, mgm./100 ml.	11.3	41.8	39.4	31.4	31.0	11.0
Serum riboflavin, μgm./ml.	0.01	1.61	1.84	1.25	1.37	0.34
Liver riboflavin, μgm./gm.	25.1	23.7	22.1	23.1	19.4	3.2
Liver riboflavin, μgm./kgm. live wt.	541	650	620	669	662	N.S.
Serum vitamin A, μgm./100 ml.	39	131	75	61	82	47
Liver vitamin A, μgm./gm.	36	40	36	29	28	N.S.
Liver vitamin A, μgm./kgm. live wt.	783	1109	1008	848	986	N.S.
Liver crude protein, %	18.4	18.2	18.3	18.0	16.7	N.S.
Liver crude protein, gm./kgm. live wt.	4.0	5.0	5.13	5.16	5.70	N.S.

* *L.S.D. = least significant difference* ($P = 0.05$).
** *N.S. = not significant.*

attain significance. Thiouracil depressed the response of serum calcium to estrogen treatment to a degree which barely failed to attain significance.

The data provided indications that thiouracil might have enhanced the increase of liver weight and of total quantity of liver crude protein induced by the estrogen treatment.

The most interesting feature of these results was the fact that thiouracil appeared to affect serum calcium and riboflavin in the estrogenized pullet in the same sense as does thyroxine, whereas thiouracil appeared to affect liver weight in the opposite sense to thyroxine.

Experiment 2

In view of the results of Experiment 1, a second similar experiment was carried out using a smaller number of pullets, but larger groups, and using thiouracil at a level of 1% in the ration. It was hoped that this experiment would provide more definite information regarding the indications suggested by the results of the first experiment.

Eighteen sexually immature Barred Plymouth Rock Pullets were assigned at random between three groups each of six pullets. The groups were given the treatments indicated in Table II. The pullets remained in excellent condition throughout the experiment. The estrogenized pullets displayed the

TABLE II

EXPERIMENT 2. EFFECTS OF THIOURACIL ON CERTAIN BIOCHEMICAL RESPONSES OF THE IMMATURE PULLET TO ESTROGEN, AVERAGE RESULTS

	Group			L.S.D.*
	1	2	3	
	No. of pullets			
	6	6	6	
Total dosage estradiol benzoate, mgm.	Nil	7 × 1.5	7 × 1.5	
Thiouracil in food, %	Nil	Nil	1.0	
Live weight, initial, kgm.	1.25	1.20	1.26	
Live weight, final, kgm.	1.37	1.30	1.36	
Total food consumption, kgm.	0.98	0.98	0.98	
Ovary weight, gm.	0.32	0.20	0.31	N.S.**
Oviduct weight, gm.	0.24	10.00	19.80	4.4
Thyroid weight, mgm.	131	110	168	33
Liver weight, gm.	25.1	28.6	40.2	3.2
Liver weight, gm./kgm. live weight	18.5	22.1	29.8	2.5
Serum calcium, mgm./100 ml.	11.1	35.8	13.6	0.6
Serum riboflavin, μgm./ml.	0.02	1.46	0.37	0.7
Liver riboflavin, μgm./gm.	29.0	25.9	23.3	3.0
Liver riboflavin, μgm./kgm. live weight	531	570	689	127
Serum vitamin A, μgm./100 ml.	44	53	43	N.S.
Liver vitamin A, μgm./gm.	37.1	42.1	22.3	N.S.
Liver vitamin A, μgm./kgm. live weight	665	906	652	N.S.
Serum protein, gm./100 ml.	3.55	5.28	3.90	0.4
Liver crude protein, %	21.1	19.2	17.8	0.17
Liver crude protein, gm./kgm. live wt.	3.9	4.3	5.3	0.27

* *L.S.D. = least significant difference* ($P = 0.05$).

** *N.S. = not significant.*

usual increase in appetite and could have eaten more food than their daily allowance. The thiouracil diet was less readily eaten during the first three days of the 14 day experimental period. No other effect of thiouracil on behavior or activity was noted. The estrogen treatments were the same as in Experiment 1 except that seven doses were administered. The pullets were 114 days old at the outset and 128 days old at the end of the experiment. The final age was greater than is desirable in such experiments, but the state of the ovaries of Group 1 at the conclusion confirmed that the birds had not approached the puberal stage.

The results of Experiment 2 are summarized in Table II. Thiouracil decreased the response of serum riboflavin to estrogen, confirming the results of Experiment 1. Thiouracil decreased the response of serum calcium to estrogen to a highly significant degree, confirming the indications secured in Experiment 1. Thiouracil decreased the response of serum vitamin A to estrogen, but this effect was not significant in the case of Experiment 2, although significant in Experiment 1.

A striking feature of the results was the fact that thiouracil brought about a highly significant increase of the hypertrophy of the oviduct of the estrogenized pullets. Fleischmann and Fried (9) noted that neither thyroxine nor thiouracil inhibited the response of the oviduct to estrogen, but they do not appear to have secured evidence that thiouracil can enhance this effect of estrogen. The data for Experiment 2 demonstrated that it is possible to produce experimentally a large degree of hypertrophy of the oviduct unaccompanied by any marked increase in serum calcium or serum riboflavin. In fact, two of the birds in Group 3 had serum calcium and riboflavin values scarcely greater than those obtained in Group 1, and yet their oviducts were strongly hypertrophied. Estimations of serum phosphoprotein phosphorus and phospholipid phosphorus were not carried out, but the appearance of the trichloroacetic acid precipitates of the sera and the behavior of the sera on dilution indicated qualitatively that the thiouracil treatment had depressed the response of serum phospholipid and phosphoprotein to estrogen as well as the response of serum calcium. The increase in total serum protein evoked by estrogen was also depressed by thiouracil, a fact which would accord with the apparent effect of thiouracil in decreasing serum phosphoprotein.

Estrogen produced a significant increase in liver weight. It is known (5, 6) that this increase is associated with an increase in the quantity of liver protein and that this is due primarily to hypertrophy of the liver cells, as shown by the relatively greater increase of ribonucleic acid than of desoxyribonucleic acid (4). In Experiment 2 thiouracil enhanced the effect of estrogen on the total amount of liver crude protein per kgm. live weight as well as the liver weight. This fact shows that the increase of weight was not merely a reflection of fatty infiltration due to toxic effects of thiouracil. The increase of liver weight was not associated with increases in serum calcium and riboflavin, but with decreases. In this regard thiouracil and thyroxine may, therefore,

decrease serum calcium and serum riboflavin by different mechanisms, for thyroxine decreases the effect of estrogen on liver weight and liver crude protein.

The differences between Groups 1, 2, and 3 in respect of concentration of riboflavin in the liver were all significant. The decreases in riboflavin concentration induced by estrogen and by thiouracil, however, must be considered in relation to the effects on liver weight. Thus estrogen by itself did not produce a significant increase in the amount of liver riboflavin per kgm. live weight in spite of its effect on serum riboflavin. It is noteworthy that thiouracil treatment superimposed upon estrogen significantly increased the μgm. liver riboflavin per kgm. live weight.

As regards liver vitamin A, Experiment 2 provided indications similar to those provided by Experiment 1, i.e., that estrogen increases the concentration of vitamin A in the liver as well as the total amount of liver vitamin A, and that thiouracil depresses this effect. The results for vitamin A did not attain statistical significance, but they are reported in Tables I and II in view of the uniform trends of the averages in both experiments.

Experiment 2 again provided indications of a slight depressant effect of estrogen on thyroid weight. Thyroid weight is a notoriously unreliable guide to thyroid activity, but in this connection it may be remarked that Epstein and Wolterink (8) have found that estrogen depresses the rate of turnover of iodine in normal chicks and in chicks made hypothyroid by thiouracil. It is possible that the tendency towards decrease in weight of the thyroid produced by estrogen in Experiments 1 and 2 is due to depression of normal growth and activity of the gland by estrogen, whereas the increase associated with thiouracil treatment is a consequence of inhibition of thyroxine production by the thiouracil, with consequent abnormal production of colloid and hypothyroidism (12).

Discussion

Fleischmann and Fried (9) suggested that thyroxine depresses serum calcium in the estrogenized chick either (*a*) by inhibiting synthesis of plasma proteins and phospholipids in the liver or (*b*) by increasing oxidative destruction of these plasma constituents. The fact that thyroxine also depresses the increase of liver weight and liver crude protein in the estrogenized pullet favors the former alternative. The effect of thyroxine would also conform with an increased rate of destruction of estrogen due to thyroxine. In the case of thiouracil, however, the depressant effect of thiouracil on serum calcium and riboflavin, as seen in the results of Experiments 1 and 2, is accompanied by enhanced oviduct and liver hypertrophy. The effect of thiouracil on the oviduct could be explained by decreased inactivation of estrogen associated with the antithyroid effect of thiouracil. The difficulty is then to explain how it comes about that this theoretical depression of estrogen inactivation does not increase serum calcium, riboflavin, etc. The theory that thiouracil interferes with inactivation of estrogen, therefore, involves the assumption that thiouracil

also interferes with the phosphoprotein and phospholipid synthesizing functions of the liver, but not with hypertrophy of the liver or with the capacity of the oviduct to respond to estrogen.

Experiments 1 and 2 provide some further support for the view that the increase of serum riboflavin in estrogenized pullets is not a reflection of mobilization of liver riboflavin (6) and that estrogen, while tending to decrease the concentration of riboflavin in the liver, also tends to increase the total amount of riboflavin in the liver. Hertz, Dhyse, and Tullner (10) have pointed out that estrogen increases serum riboflavin even when the tissues of the chick are not saturated with riboflavin, and that the magnitude of the response is related to the dietary level of riboflavin. In both experiments now reported the amounts of flavin adenine mononucleotide (FAM) and flavin adenine dinucleotide (FAD) were estimated on the sera of the estrogenized pullets by the macroadaptation of the method of Burch *et al.* (2). In all instances the amounts were too small to be measurable with any certainty in the presence of the relatively large proportion of free riboflavin. This observation tends to confirm previous suggestions (6) that the increase in serum riboflavin is primarily an increase in free riboflavin.

The results confirm previous observations that estrogen increases the level of serum vitamin A (3) and Experiment 1 provides evidence that thiouracil depresses this effect (comparison of Groups 2 and 4). The general trend of the results for the total amounts of vitamin A in the livers in both experiments suggests that estrogen tends to increase liver storage of vitamin A and that thiouracil depresses this effect. The variability of the data for liver vitamin A was such, however, that the results did not attain significance in either experiment.

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THE EFFECT OF HUMIDITY AND TEMPERATURE ON CARBON DIOXIDE PRODUCTION OF DEER MICE AND ON HEAT TRANSMISSION THROUGH THEIR FUR[1]

By J. S. Hart[2]

Abstract

The carbon dioxide production of deer mice at 1° to 18° C. was not affected by humidity, but increasing the temperature decreased the carbon dioxide production of the mice. Heat transmission of mouse fur was also independent of humidity.

Introduction

Humidity has been recognized as an environmental factor that affects heat loss from homoiotherms by modifying evaporative cooling from the lungs and skin. It may also affect heat loss by modifying the heat transmission characteristics of the fur of some animals. Rubner (8), summarizing his experiments on dogs, indicated that moist air had a twofold effect by increasing the thermal conductivity of fur and by decreasing heat loss by evaporation. An increase in humidity frequently caused a small increase in metabolism of fully fed but not of fasting dogs at cool temperatures. Murschhauser and Hidding (5) found that at 5° C. and at 20° C. guinea pigs had a greater carbon dioxide production in dry air than in saturated air, but at 35° C. the opposite occurred. They attributed the greater carbon dioxide production in dry air, at the two lower temperatures, to increased metabolism caused by greater evaporative heat loss. In rats, humidity variations at high temperatures had no effect on metabolism (3, 4). At low barometric pressure, high humidity had a beneficial effect on survival of white mice (6) and prolonged the fall in body temperature of rats (7). These beneficial effects were attributed to reduction of heat loss by evaporation. Humidity has no measurable effect on the cooling power of air as determined by dry Kata thermometer (2).

The present study was undertaken to determine the effect of humidity on metabolism (carbon dioxide production) and heat transmission of the fur of deer mice. The main emphasis was placed on the effects of humidity at low temperatures, within which heat loss by evaporation is generally low (1).

Materials and Methods

Adult deer mice, *Peromyscus maniculatus*, weighing 17–28 gm., which had been captured during the autumn in the Ottawa region, were subjected to air temperatures of 5° to 8° C. for about three weeks during February. During this period of acclimatization, seven males and eight females were kept in three groups of five. Subsequently, the five mice in each group were used simultaneously in metabolism tests.

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Carbon Dioxide Production of Mice

Carbon dioxide production was determined by the Haldane open circuit procedure. Each mouse in the group of five was confined in a separate metabolism chamber but the carbon dioxide from the five animals was pooled. A randomized working scheme was used in which each group was tested at three temperature and two humidity levels. The tests occupied 18 working days (Table I) and were arranged to minimize any effects of advancing age on the results.

The metabolism chambers were glass cylinders 8.3 cm. in diameter and 18.5 cm. long. Each was closed with a rubber stopper containing a window. A fine mesh copper cylinder 7.0 cm. in diameter was placed inside each chamber 0.5 cm. from the glass wall. The mouse was contained within a small latex-covered, cylindrical, iron wire cage that was suspended from the rubber stopper.

The chambers were immersed in a thermostatically controlled water bath. Bath temperatures were 1°, 9°, and 17° C., but, as shown in Table I, wall temperatures recorded by thermocouples on the copper cylinders near the mice exceeded bath temperatures.

Humidity was controlled by magnesium perchlorate or moist cotton between the walls of the chamber and the copper screen. Care was taken to avoid wetting the fur of the mice. In dry air tests, predried air was passed into the chambers. The moisture in the air leaving the chambers was determined gravimetrically by absorption with magnesium perchlorate. The air circulation rate was 200 to 600 ml. per minute per mouse.

Heat Transmission of the Fur

An artificial mouse was constructed as follows: The bulb of a small H-B Instrument Co. 40° C. thermostat was surrounded by a coil of resistance wire heated by a six volt battery. A small thimble of mercury surrounded the thermostat bulb. The thimble was secured to the stem of the thermostat by a rubber stopper and its outer surface was covered by the skin and fur of a mouse. The same mouse skin was used in all tests. The current supply to the thermostat was controlled by relay and the quantity of heat passing through the skin and fur was determined by an electrolytic current integrator in series with the resistance wire.

This artificial mouse was placed in a glass jar immersed in the water bath. Measurements were made while the carbon dioxide production of the deer mice was being determined. During the high humidity tests the air stream from the mice passed through the artificial mouse chamber before passing to the absorbers. In dry air tests moisture free air passed to the artificial mouse and then to the metabolism chambers. Wall temperatures of the artificial mouse chamber were obtained by placing a thermocouple on the copper screen that lined the glass chamber.

Results

Carbon Dioxide Production of Deer Mice

The carbon dioxide production of the deer mice at different temperatures and humidity conditions is recorded in Table I. Temperatures varied considerably, probably because there was an area of low heat conductivity between

TABLE I

CARBON DIOXIDE PRODUCTION OF DEER MICE (GROUPS OF FIVE) AND HEAT TRANSMISSION OF AN ARTIFICIAL MOUSE

Order test	Weight per mouse, gm.	Group No.	Wall temp., °C.	Relative humidity, %	Carbon dioxide* production	Artificial mouse	
						Wall temp., °C.	Heat transmission, cal. per hour
1	23.7	1	3.3	13.0	.0098	1.5	765
2	20.4	2	18.3	89.8	.0062	17.2	508
3	23.7	3	2.8	75.3	.0093		
4	23.5	1	10.7	9.9	.0077	9.4	640
5	22.9	2	2.7	7.5	.0012	1.6	759
6	23.4	3	10.8	80.2	.0094	9.4	648
7	23.3	1	18.4	3.7	.0065	17.3	485
8	21.7	2	11.1	84.0	.0081	9.4	652
9	24.1	3	18.0	Low	.0067	17.2	482
10	23.0	1	18.0	78.1	.0063	17.3	485
11	19.8	2	17.8	4.1	.0061	17.4	485
12	23.7	3	10.5	3.7	.0083	9.3	651
13	23.8	1	10.5	76.0	.0082	9.4	647
14	20.9	2	10.6	4.5	.0093	9.5	638
15	23.5	3	3.5	5.8	.0098	1.4	760
16	22.3	1	3.7	73.0	.0098	1.5	778
17	20.5	2	3.2	84.8	.0096	1.5	768
18	23.7	3	18.5	87.2	.0062	17.3	475

* *Expressed in gm. per hour per gm. body weight.*

the copper screen and the chamber wall. Within a single test, wall temperatures varied $\pm 0.4°$ C. Humidities also varied and fell into two groups; those below 13% and those above 73%. The recorded humidity values in high humidity tests are probably too low as condensed water was always present on the inside of the chamber window, suggesting saturation.

The average carbon dioxide production of the mice at the three temperature and two humidity levels are given in Table II. There was no significant difference in the rate of production at high and low humidities at the same temperature. The only apparent effect of the high humidity was to increase the variability of the results. The effect of temperature on the carbon dioxide production of the mice was significant but it was independent of humidity variations.

The data obtained on carbon dioxide production of the mice suggest that under certain conditions (viz., restriction of normal activity, exposure to the humidity conditions for relatively short periods, exposure to relatively cold temperatures), their metabolism is influenced by temperature but not by

TABLE II

AVERAGE CARBON DIOXIDE PRODUCTION OF DEER MICE

Wall temp., °C.	Carbon dioxide production, gm. per hour per gm. body weight			
	High humidity	Low humidity	Difference, high minus low	Average, low and low
3.2	.0093 ± .0002	.0096 ± .0001	.0003 ± .0003	.0095 ± .0002
10.7	.0081 ± .0002	.0084 ± .0001	.0001 ± .0003	.0081 ± .0002
18.2	.0064 ± .0002	.0064 ± .0001	.0000 ± .0003	.0063 ± .0002
Average	.0079 ± .0001	.0080 ± .0001	.0001 ± .0002	.0080 ± .0001

humidity. The results differ from those obtained for guinea pigs (5) for which carbon dioxide production ranged up to 14.5% greater in dry air at 5° C. and at 21° C., but they are comparable to those obtained for rats (3, 4) at high temperatures (28° to 36° C.) for which metabolism was not affected by humidity.

Heat Transmission of the Fur

Heat transmission through mouse fur and the environmental conditions of the tests are shown in Table I. Wall temperatures in these tests were lower and less variable than equivalent temperatures in the metabolism chambers. The air in the artificial mouse chamber contained, at high humidity, the same absolute quantity of water as that leaving the metabolism chambers, but at low humidity was almost free from water vapor.

The heat lost by the artificial mouse was comparable in magnitude to that estimated (from carbon dioxide production) for a single deer mouse in these tests under similar conditions. The central temperature of the artificial mouse was held at 40.0° C. The temperatures recorded under the skin averaged 32.7°, 35.4°, and 37.8° C. at the three wall temperatures which averaged 1.5°, 9.4° and 17.3° C. respectively.

The average heat transmission of the artificial mouse at the three temperature and two humidity levels is shown in Table III. Heat transmission at

TABLE III

AVERAGE HEAT TRANSMISSION OF ARTIFICIAL MOUSE

Wall temp., °C.	Heat transmission through fur, cal. per hour		
	High humidity	Low humidity	Difference, high minus low
1.5	773 ± 8.0	761 ± 2.6	12 ± 8.4
9.4	649 ± 6.5	643 ± 2.6	6 ± 7.0
17.3	489 ± 6.5	484 ± 2.6	5 ± 7.0
Average	637 ± 4.0	629 ± 1.5	8 ± 4.3

the two humidity levels did not differ significantly, although there was a general tendency for greater transmission at high humidities. Averaged over all three temperatures the difference between heat transmission at high humidity and heat transmission at low humidity was 8 ± 4.3 cal. per hour and was thus about 1.8 times the standard error.

The variability of the determinations was significantly greater at high humidities; the standard deviation of single determinations calculated from replicates at the same temperature was $\pm$ 11.4 for high humidity and $\pm$ 4.6 for low.

The physical heat transmission properties of the mouse pelt, unlike those observed for fur by Rubner, were not significantly affected by humidity. Hence unless biological properties (skin temperatures, erection of hairs, etc.) are different in moist than in dry air, there is no reason to believe that humidity has any effect on heat loss by radiation and convection. Since carbon dioxide production likewise was not significantly modified by humidity, any effect of humidity on evaporative heat loss may conceivably be counteracted by the opposite effect on radiation and convection loss. However, the interpretation of these data favored by the writer is that heat loss by evaporation, in the temperature range studied, was low and that variations in evaporative heat loss produced by humidity were relatively unimportant, considering the variability of the results. Likewise, variations in heat loss through radiation and convection were not significantly affected by humidity, in the temperature range studied, and therefore would not be expected to have any significant influence on metabolism.

Conclusions

Humidity does not have a significant effect either on carbon dioxide production of deer mice or on heat transmission through their fur at moderate and cold temperatures. Under the conditions of the experiments the effect of humidity on metabolism is unimportant compared to that of temperature.

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TIME AS A FACTOR IN THE FREEZING OF UNDERCOOLED INSECTS[1]

By R. W. Salt[2]

Abstract

Undercooling points are shown to be unreliable as a measure of insect cold-hardiness. Insects held in an undercooled state freeze at irregular intervals, often over long periods of time. Freezing, which is fatal to most insects, is initiated by the formation of an ice-crystal nucleus, and the probability of such formation is dependent upon the extent of undercooling (temperature), cold-hardiness (a complex resulting from previous treatment), and time. For a specified degree of cold-hardiness, the probability of freezing is dependent upon temperature and time. If the temperature is fixed, the probability of freezing can be expressed in units of time. Freezing can take place on a rising temperature gradient as well as on a falling one.

Unless an overwintering population is adequately protected by its environment and a high degree of cold-hardiness, losses from freezing will take place by degrees during the entire winter.

Introduction

Low winter temperatures are a limiting factor in the survival of many species of insects and often restrict their geographical range. Although the few species which hibernate in very exposed situations can survive the formation of ice in their tissues, the remainder are killed if they become frozen. The latter depend for their survival on the insulating protection of their hibernacula (soil, plant debris, snow, ice, etc.) and on their ability to undercool. The combination of these two protective factors may be sufficient that mortality as a result of freezing is a rarity in some species. More often the protection is incomplete, with the result that those individuals in the more exposed hibernacula and with the least ability to undercool perish. In severe winters this fraction may be temporarily large and may seriously deplete the population. If a species has been expanding its range into colder regions, one severe cold period can eradicate it in such areas, restricting it once again to its normal range.

A study of winter mortality can therefore be of great economic importance. There are several ways of appraising it. A census of population in the fall and in the spring is satisfactory only if all other lethal factors are known and accounted for. Exposure of insects outside during cold weather sometimes gives valuable information, but the lack of control over temperature makes this method of limited value. Entomologists have usually preferred to take their insects into the laboratory, where they control the temperature and often other factors as well. This type of work has followed two main lines, one in which the insects, usually in bulk and often in a microhabitat resembling that of their true hibernacula, are exposed to constant low temperatures for

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definite periods; and the other in which the insects are exposed individually to a falling temperature and their undercooling points recorded by a thermometer or thermocouple. It is with the latter method, and a hitherto unrecognized error, that this paper deals.

Discussion of Methods

Because ice formation is lethal, with the exception already noted, undercooling points have generally been considered precise minimum lethal temperatures. Time, in this method, is involved only in the rate of cooling and apparently affects undercooling point determinations only when the rate of cooling is very fast. At moderate rates, when the cooling takes several minutes but not more than an hour, no consistent differences in undercooling have been observed. There is, of course, a valid objection to a very rapid cooling rate: the components of the cooling apparatus, often including such a miscellany of materials as insect tissues, metal wires, glass, rubber, and air, have such diverse heat conductivities that they cannot possibly cool uniformly. This difficulty has been recognized by most workers, who used rates of cooling slow enough that the undercooling points were *apparently* unaffected.

It is clear, however, that the adjectives *slow* and *fast* as used in such laboratory tests are not comparable with those experienced in nature. Winter cold is reckoned in larger time units—hours at least, and more often days, weeks, or months. The influence of such long periods has received little or no attention except as it affects the hardening process (often resulting in lowered undercooling points and hence greater cold resistance). It has been assumed, intentionally or otherwise, that time spent in the undercooled state has no harmful effect (from the temperature standpoint alone; dehydration and depletion of metabolic reserves gradually become lethal), and only when the undercooling point is reached is the insect endangered. The action of contact moisture in reducing the degree of undercooling does not change the mortality picture; freezing is fatal.*

It has been generally accepted that each individual insect possesses a definite undercooling point, predetermined by such things as species, stage, nutrition, moisture, and temperature, but nevertheless fixed for any set moment. This would be the normal undercooling point as determined in the laboratory. No means is known of rapidly increasing the degree of undercooling, but it can be decreased by inoculation of the crystallization process with contact ice, by injury of the specimen in such a way as to rupture the integument, or by mechanical disturbance such as shaking, bending, or prodding. Each of these methods works most readily when undercooling is greatest and becomes gradually less effective as the temperature is raised.

** Some insects apparently survive freezing at a high temperature, as when contact moisture has reduced undercooling to a few degrees; but it has been the writer's experience that they are always adversely affected and their death is only a matter of time, even though they may appear normal for some time after thawing.*

In the present work, moving the specimens while they were in the cold rooms was avoided as much as possible. Prodding them to test for hardness was necessary in some species, particularly at the higher temperatures, whenever appearance alone was not sufficient to indicate the frozen condition. Because of the long exposure periods involved, it became necessary to protect the material against desiccation. Covered Petri dishes were used at first; later these were placed in large covered culture dishes over ice. To determine whether atmospheric moisture influenced freezing, precooled sawfly larvae were placed in two covered dishes at −20° C., one containing saturated air over ice and the other dry air over calcium chloride. The course of freezing was virtually the same in the two groups.

Experimental Results

Mature larvae of the wheat stem sawfly, *Cephus cinctus* Nort., were found to have undercooling points lying predominantly between −20° and −30° C. The lowest undercooling point recorded for 200 larvae was −32.7° C.; at the upper extremes the data were suspect, as chance injury or condensation can raise the undercooling points considerably. Including doubtful cases, however, only 4.5% froze above −20° C., 3.0% above −19° C., 2.0% above −17° C., and 1.5% above −15° C. In other words, about 95% should remain unfrozen when exposed to −20.0° C. The question arises: how long will they remain unfrozen? Larvae were placed under observation in a series of constant temperature rooms at −5°, −10°, −15°, and −20° C., all ±0.5° C., and in a refrigerated cabinet at −27°, ±0.5° C.

At −20° C. frozen larvae of the wheat stem sawfly are readily distinguishable by their white coloration, contrasting with the oily straw coloration, of unfrozen larvae. If watched closely, the actual freezing can be observed. A congelation wave sweeps along the larva in less than a second, producing a whitening that intensifies during the next half-minute.

Twenty mature larvae of *Cephus cinctus* were exposed to −20° C. in a small Petri dish whose lid was left off for the first hour to avoid moisture condensation. In 15 min. one larva was frozen; in 30 min., three; in one hour, four. In the next hour no more froze, but overnight five more did. None froze during the next day, two froze during the second night, and one the following morning. In a 48-hr. period 12 had frozen. The remaining larvae froze on the 5th, 9th, 11th, 19th, 22nd, 25th, and 82nd days.

A second series at −20° C., of 18 larvae, was more resistant. None froze during the first two days, one on the third, two on the fourth, and one each on the 5th, 8th, 13th, 15th, 16th, 28th, and 33rd days. On the 42nd day two froze, one on the 66th day, and one on the 71st day, making a total of 14 larvae out of 18. The remaining four larvae were still unfrozen after 120 days. In a third series, less resistant, all had frozen by 26 days at −20° C.

Further tests gave fundamentally similar results. Larvae of the wheat stem sawfly were used for the most part because of suitability in size, color, softness, availability, and freezing range. Also tested were diapause eggs

of the clear-winged grasshopper, *Camnula pellucida* (Scudd.); overwintering larvae of the beet webworm, *Loxostege sticticalis* (L.); overwintering larvae of the goldenrod gall, *Eurosta solidaginis* (Fitch); feeding larvae of the pale western cutworm, *Agrotis orthogonia* Morr.; and pupae of *Cephus cinctus* Nort. All of these reacted in essentially the same manner as larvae of the wheat stem sawfly. There were, of course, differences in cold resistance among species, among groups of the same species, and among individuals. Any factor that influences cold-hardening will cause such variations. In addition, the element of chance in the formation of ice-crystal nuclei adds one more variable (see discussion) and rules out reproducible results. For these reasons the data presented graphically in Figs. 1 and 2 should be interpreted not in a strictly quantitative sense but rather to show the influence of time on mortality at a constant subfreezing temperature.

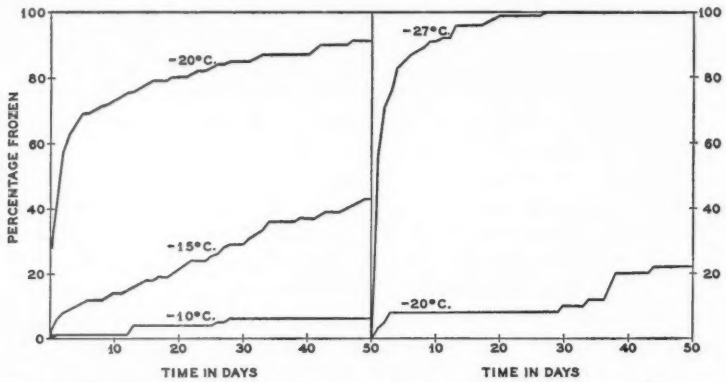

FIG. 1. *Sample mortality curves of larvae of* Cephus cinctus *exposed to −20°, −15°, and −10° C.*

FIG. 2. *Sample mortality curves of diapause eggs of* Camnula pellucida *exposed to −27° and −20° C.*

Supplementing Figs. 1 and 2, which are relatively short-term, Table I shows that freezing takes place in larvae of *Cephus cinctus* over a five-month period at −15° C.

TABLE I

PERCENTAGE OF *C. cinctus* LARVAE THAT FROZE WHEN EXPOSED TO −15°.C. (LARVAE INTACT IN THEIR STUBS,* WHICH WERE COVERED WITH MODERATELY MOIST SOIL)

Exposure period, days	% Frozen	Exposure period, days	% Frozen
100	78	140	96
110	88	150	98
120	82	160	98
130	97	200	100

** The term stub applies to the base of the stem which remains after the larva has girdled the stem just above the soil surface. The larva plugs the end of the stub with frass, lines it with a membranous cocoon, and spends the winter and following spring in it.*

By freezing weighed sawfly larvae it was also established that there was no relationship between weight and the order in which the larvae froze.

Discussion

An undercooled liquid is in an unstable state. If a crystal of the solid phase is introduced into the system, crystallization will proceed with the crystal as nucleus. If no such crystal is introduced, crystallization must start with undercooled liquid molecules, presumably oriented by chance into a configuration that favors the change of phase. Little is known about this process, but once the crystal is started its growth is assured; if the system is water–ice, growth of the crystal is very rapid. At present we are concerned with the origin of the first crystal and especially with the factors that contribute to its formation.

It is known that undercooling of the body fluids is the rule in insects. In fact, it is difficult to eliminate undercooling in an insect; one can reduce it, but it may be virtually impossible to eliminate it. Many insects normally undercool 20 to 30 Centigrade degrees, and some are able to undercool considerably more for a short time. The theory of crystal nucleus formation and experimental work lead us to believe that, for undercooled water within the limits found in nature (say above −50° C.), the greater the undercooling the more chance there is that a crystal nucleus will form.* Time automatically enters as a factor because the chances of such an event happening are related to time. The experimental results reported here support this concept.

Take, for example, the freezing of a larva of the wheat stem sawfly that is more resistant than most of its fellows. If it is cooled in a rubber and glass thermocouple holder, cooling from +20° to −30° C. in a matter of half an hour or less, the undercooling point may be, let us say, −30.0° C. The same insect, placed at a constant temperature of −25° C., would probably freeze only after a few hours or days; at −20° C., only after a few weeks; and at −15° C., only after several months. The time required for a crystal nucleus to form in such a specimen would therefore be a matter of seconds at −30° C., hours or days at −25° C., weeks at −20° C., and months at −15° C. A less cold-hardy specimen would require correspondingly less time at each temperature except −30° C., which it could not attain at all in an unfrozen state.

Presently accepted methods of determining undercooling points are faulty because they provide a falling temperature gradient that steadily increases the chances of crystal nucleus formation. Ultimately the chances become overwhelming, i.e., the time factor approaches zero. This represents the *limit* of undercooling for a particular case, but it should be particularly noted that freezing *could* have occurred previously at a higher temperature, though the probability of this event was much smaller.

Although all insects are probably subject to the same fundamental influences when undercooled, they differ in cold resistance, which is merely their ability

** Below −50° C., or thereabouts, the chances of crystallization become rapidly less, and the phase change is to the vitreous state (1).*

to remain undercooled without freezing. The variability can be explained by assuming that the colloids, electrolytes, and such, associated with body water, influence the chances of nucleus formation. For example, a comparatively cold-resistant insect would have a lower probability of ice-formation than a less cold-resistant insect at the same sub-freezing temperature. The prevailing method of undercooling point determinations would, it is true, establish the same relationship, but it lacks practical significance because the cooling rates are unnaturally fast. Existing data on undercooling points represent maximum resistance to cold and as such are of some value. But the practical entomologist is concerned with the whole story; he will need to investigate the increase in mortality during the entire winter.

Those workers who determined cold-resistance by exposing insects to a series of time–temperature combinations probably obtained valid data within the limits of time and temperature that they used. Many of them found that mortality increased with time at a given temperature, and in some of these cases mortality was definitely restricted to freezing. It is surprising that the importance of time in the initiation of freezing, as distinguished from its lethal effects, was not studied and interpreted more carefully. To be adequate, laboratory studies on winter mortality should henceforth cover the entire time–temperature range from the temperature where the time required to freeze all specimens is only a matter of a few minutes up to one where at least some freezing occurs during a period of four or five months. They should also take into consideration the pretreatment of the material insofar as it affects hardening. To be of much practical value, the work should be conducted on material that is as cold-hardy as that which could be expected under natural conditions.

The literature on winter mortality in insects naturally contains a number of anomalies which can now be explained. For example, Salt (3) found that larvae of the common cattle grub, *Hypoderma lineatum* (De Vill.), had undercooling points below −19° C. and concluded that they were safe at any temperature above this. Pfadt (2) obtained frozen (and killed) larvae of the same species after eight hours at −10° C. and after one, four, and eight hours at −15° C. Had he kept them longer at these temperatures, the proportion of frozen specimens would undoubtedly have been greater. Though incomplete, Pfadt's findings are much closer to the practical information that both writers sought. In numerous other cases in which undercooling point ranges have been interpreted as lethal temperature limits, it will be found that the insects have much less protection against cold than was formerly thought. Whereas we have been accustomed to the idea that successively lower temperatures are required to keep adding to winter mortality, it is now apparent that any subfreezing temperature can become lethal in time and that freezing will occur on a rising temperature gradient as well as on a falling one.

Overwintering insect populations are, of course, in no greater danger than they ever were. Many species undoubtedly possess sufficient environmental protection and inherent cold-resistance so that they never suffer any mortality

from freezing. The wheat stem sawfly, *Cephus cinctus* Nort., is in this class or very nearly so. It has been used extensively for experimental work at the Lethbridge laboratory for 20 years and is collected by the thousands each spring. Although larvae that had been frozen would be readily detected, they have not been found in noticeable numbers. Other species are less fortunate. Their cold-resistance and their environmental protection are together not sufficient to protect the entire population against freezing. Though they are never in danger of eradication, their numbers are continually reduced by subfreezing winter temperatures.

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